Amendments to the Claims/Listing of Claims

Please amend claims 1, 42 and 43 as follows. This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently amended) A method for **improving success of achieving** crystallographic structure determination of a protein, said method comprising:

(a) identifying at least one unstructured region of said protein by hydrogen exchange analysis;

(b) deleting at least one unstructured region of said protein identified by hydrogen exchange analysis; and

(c) subjecting to crystallization and structure determination one or more modified form(s) of said protein generated by deleting at least one unstructured region of said protein~~, wherein said at least one unstructured region is identified by hydrogen exchange analysis~~.

2. (Original) A method according to claim 1, wherein said hydrogen exchange analysis comprises determining the quantity of isotopic hydrogen or the rate of hydrogen exchange, or both the quantity of isotopic hydrogen and the rate of hydrogen exchange, of a plurality of peptide amide hydrogens exchanged for said isotopic hydrogen in a protein labeled with a hydrogen isotope other than 1H.

3. (Original) A method according to claim 2, wherein said determining the quantity of isotopic hydrogen or the rate of hydrogen exchange or both the quantity of isotopic hydrogen and the rate of hydrogen exchange comprises:

(a) fragmenting said labeled protein into a plurality of fragments under slowed hydrogen exchange conditions;

(b) identifying which fragments of said plurality of fragments are labeled with isotopic hydrogen;

(c) progressively degrading each fragment of said plurality of fragments to obtain a series of subfragments, wherein each subfragment of said series is composed of about 1-5 fewer amino acid residues than the preceding subfragment in the series; and

(d) correlating the amount of isotopic hydrogen associated with each subfragment with an amino acid sequence of said fragment from which said subfragment was generated, thereby determining the quantity of isotopic hydrogen or the rate of hydrogen exchange or both the quantity of isotopic hydrogen and the rate of exchange of a plurality of peptide amide hydrogens exchanged for said isotopic hydrogen in a protein labeled with a hydrogen isotope other than 1H.

4. (Original) A method according to claim 3, wherein said fragmenting in step (a) comprises contacting said labeled protein with at least one acid stable endopeptidase.

5. (Original) A method according to claim 4, wherein said at least one acid stable endopeptidase is coupled to a support material.

6. (Original) A method according to claim 5, wherein said at least one acid stable endopeptidase is selected from the group consisting of pepsin, cathepsin D, newlase, Aspergillus proteases, thermolysin, protease type XIII, and combinations of any two or more thereof.

7. (Original) The method according to claim 6, wherein said at least one acid stable endopeptidase is pepsin.

8. (Original) A method according to claim 3, wherein said progressively degrading comprises contacting said fragments with at least one acid stable exopeptidase.

9. (Original) A method according to claim 8, wherein said at least one acid stable exopeptidase is coupled to a support material.

10. (Original) A method according to claim 9, wherein said progressively degrading comprises contacting said fragments with at least one acid resistant carboxypeptidase.

11. (Previously presented) A method according to claim 10, wherein said at least one acid resistant carboxypeptidase is carboxypeptidase P.

12. (Original) A method according to claim 9, wherein said fragmenting in step (a) comprises contacting said labeled protein with at least one acid stable endopeptidase selected from the group consisting of pepsin, newlase, cathepsin C, Aspergillus proteases, protease type XIII, thermolysin, and combinations of any two or more thereof.

13. (Original) A method according to claim 2, wherein said determining the quantity of isotopic hydrogen or the rate of hydrogen exchange or both the quantity of isotopic hydrogen and the rate of exchange comprises:

(a) generating a population of sequence-overlapping fragments of said labeled protein by treatment with at least one endopeptidase under conditions of slowed hydrogen exchange, and then

(b) deconvoluting fragmentation data acquired from said population of sequence-overlapping endopeptidase-generated fragments.

14. (Original) A method according to claim 13, wherein said population of sequence-overlapping endopeptidase-generated fragments is generated by cleaving said protein with an endopeptidase selected from the group consisting of a serine endopeptidase, a cysteine endopeptidase, an aspartic endopeptidase, a metalloendopeptidase, a threonine endopeptidase, and combinations of any two or more thereof.

15. (Original) A method according to claim 13, wherein said at least one endopeptidase is coupled to a support material.

16. (Original) A method according to claim 13, wherein said at least one endopeptidase is pepsin.

17. (Original) A method according to claim 13, wherein said population of sequence-overlapping endopeptidase-generated fragments is generated by two or more endopeptidases used in combination.

18. (Withdrawn) A method according to claim 13, wherein said at least one endopeptidase is newlase or Aspergillus protease XIII.

19 (Withdrawn) A method according to claim 13, wherein said at least one endopeptidase is an acid-tolerant Aspergillus protease.

20. (Original) A method according to claim 13, wherein said population of sequence-overlapping endopeptidase-generated fragments is generated at a pH of about 1.8 - 3.4.

21. (Original) A method according to claim 20, wherein said population of sequence-overlapping endopeptidase-generated fragments is generated at a pH of about 2 - 3.

22. (Original) A method according to claim 21, wherein said population of sequence-overlapping endopeptidase-generated fragments is generated at a pH of about 2.0-2.5.

23. (Original) A method according to claim 21, wherein said population of sequence-overlapping endopeptidase-generated fragments is generated at a pH of about 2.5 - 3.0.

24. (Original) A method according to claim 13, wherein said population of sequence-overlapping endopeptidase-generated fragments is generated in less than five minutes.

25. (Original) A method according to claim 24, wherein said population of sequence-overlapping endopeptidase-generated fragments is generated in about one minute or less.

26. (Original) A method according to claim 25, wherein said population of sequence-overlapping endopeptidase-generated fragments is generated in about 40 seconds or less.

27. (Original) A method according to claim 13, wherein deconvoluting comprises:

comparing the quantity of isotope and/or rate of exchange of hydrogen at a peptide amide hydrogen with said isotope on a plurality of endopeptidase fragments in said population of sequence-overlapping endopeptidase-generated fragments with the quantity of isotope and/or rate of exchange of hydrogen at a peptide amide hydrogen on at least one other endopeptidase fragment in said population of sequence-overlapping endopeptidase-generated fragments,

wherein said quantities are corrected for back-exchange losses subsequent to the initiation of slowed exchange conditions in an amino acid sequence-specific manner.

28. (Original) A method according to claim 27, wherein labeled peptide amides are localized in an amino acid sequence-specific manner by measuring rates of exchange as a function of time under slowed exchange conditions.

29. (Original) A method according to claim 13, wherein said population of sequence-overlapping endopeptidase-generated fragments contains a plurality of sequence-overlapping fragments, wherein more than half of the members of said population have sequences that overlap other members of said population over all but 1-5 amino acid residues.

30. (Original) A method according to claim 13, wherein a majority of members of said population of sequence-overlapping endopeptidase-generated fragments is present in an analytically sufficient quantity to permit its further characterization.

31. (Original) A method according to claim 13, wherein determining the quantity and rate of exchange of peptide amide hydrogen(s) is carried out contemporaneously with generating a population of sequence-overlapping endopeptidase-generated fragments.

32. (Original) A method according to claim 13, further comprising determining off-exchange rates of labeled peptide amides under conditions of slowed hydrogen exchange and random-coil conditions from a plurality of fragments and fragment differences.

33. (Original) A method according to claim 3 or claim 13, wherein said isotopic hydrogen is deuterium.

34. (Original) A method according to claim 33, wherein the presence and quantity of said deuterium on said fragments of said labeled protein is determined by measuring the mass of said fragments.

35. (Original) A method according to claim 34, wherein said measuring is performed using mass spectrometry.

36. (Original) A method according to claim 3 or claim 13, further comprising the use of conditions that effect protein denaturation under slowed exchange conditions prior to generation of said fragments.

37. (Original) A method according to claim 36, wherein said conditions comprise contacting said labeled protein with guanidine hydrochloride at a concentration of about 0.05 - 4 M.

38. (Original) A method according to claim 36, wherein said conditions comprise contacting said labeled protein first with guanidine thiocyanate at a concentration of about 1.5 - 4 M, followed by dilution into guanidine hydrochloride at a concentration of about 0.05 - 4 M.

39. (Original) A method according to claim 3 or claim 13, further comprising disrupting disulfide bonds in the labeled protein prior to generating said fragments.

40. (Original) A method according to claim 39, wherein said disrupting comprises contacting the labeled protein with a phosphine.

41. (Original) A method according to claim 1, wherein said protein is a protein that is resistant to crystallization or that forms crystals that do not diffract X-rays sufficient for structure determination.

42. (Currently amended) A method for **improving success of achieving** crystallographic structure determination of a protein, said method comprising :

(a) generating one or more modified forms of said protein by deleting at least one unstructured region of said protein identified by hydrogen exchange analysis; and

(b) subjecting to crystallization and structure determination said one or more modified forms of said protein.

43. (Currently amended) A method for **improving success of achieving** crystallographic structure determination of a protein, said method comprising :

(a) identifying unstructured regions in the said protein by hydrogen exchange analysis;

(b) generating one or more modified forms of said protein by deleting at least one of said unstructured regions of said protein; and

(c) subjecting to crystallization and structure determination said one or more modified forms of said protein.

44. (Withdrawn) A method for crystallographic structure determination of a protein, said method comprising :

(a) selecting a protein that is resistant to crystallization or that forms crystals that do not diffract X-rays sufficient for structure determination;

(b) identifying unstructured regions in the said protein by hydrogen exchange analysis;

(c) generating one or more modified forms of said protein by deleting at least one of said unstructured regions of said protein; and

(d) subjecting to crystallization and structure determination said one or more modified forms of said protein.

45. (Withdrawn) A method of refining a crystallographic structure determination of a protein of interest, said method comprising:

comparing an initial crystallographic structure determined using crystal(s) of said protein to at least one other crystallographic structure determined using crystal(s) of at least one modified form of said protein,

wherein said modified form(s) of said protein is(are) obtained by identifying and deleting at least one unstructured region of said protein or a portion thereof using hydrogen exchange analysis.

46. (Withdrawn) A method of crystallization of a protein of interest, said method comprising:

comparing an initial hydrogen exchange stability map of said protein to at least one other hydrogen exchange stability map of at least one modified form of said protein, wherein said modified form(s) of said protein is(are) obtained by identifying and deleting at least one unstructured region or a portion thereof of said protein, and

subjecting to crystallization one or more modified form(s) of said protein exhibiting an improved hydrogen exchange stability map.

47. (Withdrawn) A method of crystallographic structure determination of a protein of interest, said method comprising:

comparing an initial hydrogen exchange stability map of said protein to at least one other hydrogen exchange stability map of at least one modified form of said protein, wherein said modified form(s) of said protein is(are) obtained by identifying and deleting at least one unstructured region or a portion thereof of said protein, and

subjecting to crystallization and structure determination one or more modified form(s) of said protein exhibiting an improved hydrogen exchange stability map.

48. (Withdrawn) A method of characterizing conformational differences between a protein in solution and the same protein in crystal form, said method comprising:

comparing a characterization of said protein in solution to a characterization of said protein in crystal form;

wherein said characterization of said protein in solution is obtained by hydrogen exchange analysis,

and wherein said characterization of said protein in crystal form is obtained by hydrogen exchange analysis after incubating said protein in crystal form in a microcrystalline suspension in deuterated water, under conditions where dissolution of the protein in crystal form is inhibited.